LIFE CYCLE STUDY OF MALARIA VECTOR *ANOPHELES ACONITUS* DONITZ IN THE LABORATORY

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ABSTRAK

Anopheles aconitus Donitz, merupakan vector utama penyakit malaria di daerah-daerah sekitar persawahan di pulau Jawa, sejak tahun 1983 telah berhasil dikembangbiakkan di laboratorium. Siklus hidupnya dari telur sampai dewasa paling singkat selama 11 hari, sedang umumnya antara 13 sampai 16 hari. Perkawinan terjadi sebelum nyamuk mengisap darah. Nyamuk mulai menghisap darah pada dua hari setelah muncul dari pupa dan bertelur dua sampai lima hari kemudian. Setelah bertelur nyamuk akan menghisap darah lagi. Dari munculnya nyamuk dewasa sampai bertelur yang pertama diperlukan waktu antara empat sampai tujuh hari, sedang peletakan telur berikutnya terjadi paling cepat dua hari dan paling lama lima hari setelah menghisap darah. Nyamuk generasi baru akan muncul setiap 15 hari sekali. Nyamuk jantan maupun betina dapat bertahan hidup sekitar 25 hari, sekitar 50% nyamuk jantan hidup lebih dari 13 hari dan nyamuk betina lebih dari 12 hari.

INTRODUCTION

Anopheles aconitus Donitz, the most important malaria vector in rural areas of Java (Sundararman et al., 1957) breeds mainly in terraced rice fields where clear water continuously flows slowly from one field plot to another, and to a lesser degree in irrigation canals and margin of streams (Sundararman *et al.*, 1957 and Joshi *et al.*, 1977). Intensive observations on the extent of larval breeding in ricefield showed that paddy nurseries are free from *An. aconitus* larvae and the breeding starts approximately two to three weeks after the transplantation of seeding from the nurseries to the field (Sundararman *et al.*, 1957). Adult mosquitoes are found throughout the year and the population densities are influenced by rainfall and stages of rice growth.

The distribution of this species is from the coastal plains at sea level to the central plateau up to an altitude of about 1000 meters (Sundararman *et al.*, 1957).

The colonization of this species has been attempted in the past, but without success. A break-through was made in 1983 and at least 20 generations have been produced without induced copulation. Subsequent colonization was again carried out and since then this species has been well established in laboratory conditions. This paper presents the life cycle of *An. aconitus* and procedure for

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handling all stages of development in the laboratory.

MATERIAL AND METHODS

Source of An. aconitus

1. Wild caught females.

An. aconitus blood fed and gravids were collected from cattle shelters and natural outdoor resting places (along stream banks and in vegetation along irrigation ditches) in Kaligading village, Kendal regency, Central Java. This village is located ± 30 km south west of Semarang, at elevation of ± 400 meters. Mosquitoes were collected in the morning by mouth aspiration. These mosquitoes were transported from the field to the laboratory in unwaxed paper cups and were placed in wooden mosquito carrying boxes covered with damp towels to maintain its adequate humidity. Each paper cup containing a maximum of 40 mosquitoes.

2. Colony.

The colony consists of several generations of laboratory reared *An. aconitus*. Cages of 35 x 35 x 70 cm^3 were used to rear a 10.000 to 15.000 mosquitoes. Earthen pots 11 cm in diameter and 11 cm high, one-third filled with water from well, were placed in the cages to provide sites for egg deposition. The colony produced eggs daily.

Oviposition.

Wild caught mosquitoes from the field were transfered into monocups for oviposition. These cups were line with wet filter papers at the bottom and sides, the bottoms of the cups were also provided with a thin layer of wet cotton under the filter paper. The tops of the cups were also provided with a 10% sugar solution soaked in cotton. These cups were then returned to the carrying boxes for holding. To maintain high humidity the boxes were covered with moist small towels. The cups were checked daily for eggs.

In order to observe the number of eggs produced, the females were placed individually reared in small bottles (3 cm in diameter and 5 cm high). The bottoms of the bottles were provided with a thin layer of wet cotton and lined with wet filter papers. The tops of the bottles were also provided with cotton soaked in 10% sugar solution.

Egg hatching and handling of larvae.

Wet filter papers containing eggs from the wild caught and eggs from earthen pots produced by the colony were transfered into enamel pans (35 x 24 x 5 cm). These pans contained about 700 ml of well water with a pH ± 7. A 4 cm strip of filter paper was placed along the inner walls of the pans to prevent egg desiccation. Papers were removed when eggs were free floating.

No food was given to the first instar larvae on the first day. On the second day and onward water was changed, larvae was exposed to sun light for 10 to 15 minutes while food was provided. If the larvae were too crowded in the pans they were divided into additional pans by pippeting or dipping with a large spoon.

Larval food was prepared containing one part of powdered lean dried beef, five parts of commercial dry dog's food, which has a low fat con-

tent (Gaines Meals^{R}) and ten parts of yeast filtered through a cloth. The feeding was geared with the development of the larvae and the amount given as follow :

Day after hatching	*Weight of food (mg)*
2 — 3	100
4 — 5	300
6 — 7	400
8 — 10	500

After 10 days the amount of food was given sufficiently (approximately 1 mg per larvae), because most of the larvae had become pupa. Food was sprinkled, little by little, on the surface of water. A box fan was operated for aeration.

Handling of pupae.

The pupation did not occur simultaneously. Pupae were transfered daily into enamel bowls containing water by pippeting and then exposed into sunlight for 10 to 15 minutes. Afterwards pupae were put into mosquito cages.

Handling of the adults.

Cage of 35 x 35 x 35 cm^3 were used to rear a maximum of 5000 mosquitoes and 35 x 35 x 70 cm^3 for 10,000 to 15,000 mosquitoes. Mosquito cages were covered with wet towels to regulate humidity and temperature within the cages. To maintain temperature and high humidity in the room the windows were opened all the time, plastic buckets (45 x 30 x 15 cm^3) half filled with water and hollow bricks half soaked in the buckets, were placed in the insectary. The temperature of the room ranged from 23 to 32°C and relative humidity 58 to 85%.

Male mosquitoes were provided cotton pads wetted with 10% sugar solution and raisins. The second day after the adults' emergence and onward, a guinea pig is provided for blood feeding. Earthen pots filled with water were placed in the cage for oviposition and another earthen pot full with water, covered with net provided for resting place.

Determination of sex ratio

Sex ratio was determined by rearing pupae in monocups filled with water. About 2,000 pupae were put into 20 monocups and the top of each monocup was covered with a mosquito net. The emerging of adults were counted and identified daily until all pupae turned into adults.

Determination of oviposition.

Blood fed females were observed after the first blood meal until the first egg laying. About 1000 pupae were put into a cage, with four replications.

The first cage was blood-fed one day after all pupae turned into adults and then after laying eggs stopped, the mosquitoes were again blood-fed. The second cage was blood-fed two days after all pupae turned into adults and after laying eggs stopped, they were blood-fed again. The third cage was blood-fed three days after all pupae turned into adults and next blood-fed was given at the day after mosquitoes stopped laying eggs. The fourth cage was blood-fed one day after all pupae turned into adults and continued blood fed every day.

Determination of lor.gevity.

About 1,000 pupae were put in cage of 35 x 35 x 35 cm^3 and two days and onward after all pupae turned into adults was blood-fed. Dead mosquitoes were removed from the cage daily by mouth aspiration, counted and identified to sex until all the mosquitoes died. Four cages were used in this study for replication.

RESULTS AND DISCUSSION

Gravid and fed *An. aconitus* collected from the field laid eggs in one to three days after transferring them into monocups and small bottles. Percentage of mosquitoes laying eggs in small bottles were about 29% from 147 mosquitoes. The amount of eggs laid by each mosquito varied from two to 168 eggs with an average of 91 eggs per-mosquito; and 57% laid eggs more than average. Dissection of dead gravid mosquitoes before laying eggs showed about 89 to 213 rudimentary eggs, or an average of 128 rudimentary eggs per-female in 27% of the female mosquitoes examined. The result showed that the number of eggs deposition in each female varies. The differentiation of eggs deposition was also found in other Anophelinae (Roy, 1921 and Datinove, 1955 in Clement, 1963). Their observation showed positive correlations among the number of eggs deposition in larger female mosquitoes and the volume of blood meals.

The eggs hatched in two days after putting them into water and the amount of eggs hatched was about 70%. The larvae passed through four instars and the development of each instar took place every two to three days. The process of pupation is shown in Table 1. The first pupation occurred 7 or 8 days after the egg hatching, but still low, about 0,5 to 10,2%. Most of the pupation occurred at 9 to 12 days.

The larval mortality were very low, about 0 to 14.6% in the pans filled with 200 to 800 larvae, while pans filled with 1000 or more larvae, the mortality rate increased to about 30%, and the development of the larvae also took longer time to develop.

The duration of the pupae stages was two days and adults emerged two days yielding. The result of care of the pupae is shown in Table 2.

The mortalities of the pupae were low ranging from 1.8 to 5.5 % with an average of 3.5% from 3991 pupae. The proportion of female to male in each month was not the same. Table 2 shows that pupae came from dry season generation (September, with rainfall 2,5 mm) yield adult with a proportion female to male, about 2 to 1, while pupae came from rainy season generation yield adults with a proportion 1 to 1. This result indicates that there were movements of sex ratio on *An. aconitus* population during dry season to rainy season. Sunjaya (1970) stated that the season is one of the factors influential to the movement of sex ratio. Generally, the proportion of female and male on the eggs fertilization time is about 50% to 50% and mortality on both sexes during embryonal development was due

Tabel 1. Percentage of larval mortality and of pupation after eggs hatching from various generation

Generation/ Colony	Larva Number	Mortality (%)	Percentage of the pupation at the day after eggs hatching											Average (days)
			7	8	9	10	11	12	13	14	15	16	17	
Wild caught	315	2,5		10,2	15,9	35,9	23,8	11,7	—	—	—	—	—	10
	644	3,4			4,7	28,0	43,9	16,8	1,7	0,5	—	—	—	11,5
	1232	30,0	0,8	3,2	8,5	12,3	17,0	16,1	7,0	3,7	0,5	0,1	0,1	12
10^{th} generations	272	0,0		7,3	51,4	42,0	5,9	—	—	—	—	—	—	9,5
	426	2,6	0,5	1,4	61,3	32,4	3,3	0,6	1,1	—	—	—	—	10
	797	7,4			0,6	8,4	22,1	43,0	15,7	2,5	—	—	—	11,5
Colony	200	3,0			36,0	56,0	1,5	2,5	1,0	—	—	—	—	11
	307	4,7		5,9	29,3	42,3	11,4	4,9	1,6	—	—	—	—	10,5
	550	14,6		2,4	56,6	23,6	1,8	0,7	0,9	—	—	—	—	10,5
	761	14,4		1,4	18,3	56,6	6,2	1,2	1,2	0,3	0,4	—	—	11

Table 2. Percentage of pupae mortalities and the proportion of male and female mosquitoes from September 1983 to January 1984.

Month/Year	Pupae		% of An. aconitus					Sex ratio	Rainfall
	No.	% mort. 1)		Male 2)		Female 2)			
Sept. '83	—	—		—		—		—	2.50
Oct.	998	2,80	(28)	29,50	(294)	67,70	(676)	1,0 : 2,3	513
Nov.	988	1,80	(18)	41,70	(412)	56,50	(558)	1,0 : 1,4	290
Jan. '84	1005	2,10	(21)	54,40	(547)	34,50	(437)	1,3 : 1,0	391
Feb.	1000	5,50	(55)	49,30	(493)	45,20	(452)	1,1 : 1,0	342
Total	3991	3,51	(122)	43,70	(1746)	53,20	(2123)	1,0 : 1,2	

1) Number of pupae died (in bracket)
2) Number of adult mosquitoes (in bracket)

to seasonal influence.

Table 3 shows that *An. aconitus* started blood meals on the second day after emerging from the pupae and the minimum time of eggs laying occurred in the second day with a maximum of five days after blood meals. Mating took place before blood meals. According to Curtin and Jones study in 1961 reported by Clements (1963), mature eggs hatched almost simultaneously. But from our study mosquitoes did not lay the eggs at the same time. The minimum from the mosquitoes emerging to the first laid eggs was about 4 days and the maximum was about 7 days, while the next ovipositions occurring every 2 to 5 days after blood meals. In the laboratory conditions *An. aconitus* only laid eggs three times. Ovary dissection of the mosquitoes of 20 days old mosquitoes also showed with three dilatations only.

Table . The oviposition cycle of An. aconitus in different time of feeding.

Number of Pupae	Days after emergence																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1000	XX	—	—	XX	—	00	00	00	00	XX	—	00	00	00	00	XX	—	00	—	—	—	—	—	—
1000	—	XX	—	00	00	00	00	XX	—	00	00	00	XX	—	XX	—	—	—	—	—	—	—	—	—
1000	—	—	XX	—	00	00	—	XX	—	00	00	00	00	—	XX	—	—	—	—	—	—	—	—	—

XX : Feeding time
00 : Eggs laying
— : No eggs laying

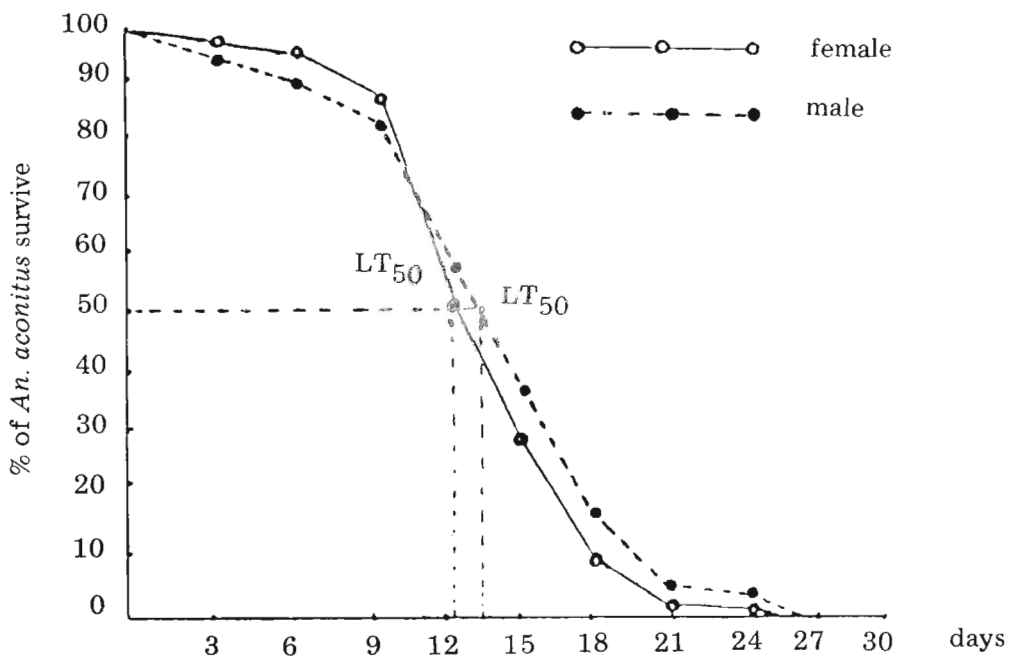

Figure 1. The longevity of female and male *An. aconitus*

In the laboratory condition male and female mosquitoes can survive for about 25 days, when blood meals and sugar water are continuously available (Figure 1). About 50% of the female mosquitoes survived for 12 days and 50% of the males survived for 13 days.

CONCLUSION

The cycle of malaria vector, *An. aconitus* from egg to adult is about 12 days. Mating occuring before blood meals. Blood feeding started second day after emergence and egg laying 2 to 5 days later. The minimum time of *An. aconitus* from emergence to the first egg laying was 4 days. The next blood feeding occuring after eggs laying and the next egg laying varies from 2 to 5 days.

The incubation period of egg stages was 3 days, the minimum time of larval stage was 8 days, generally 10 days and pupae for 2 days. In the laboratory condition with the temperatures range from 23°C to 32°C and relative humidity 50% the emergence of new generation was at every 16 days. The minimum time of gonotrophic cycle took place every 2 days and maximum 5 days. The maximum survival period for female and male mosquitoes was 25 days. Fifty percent survived 12 days for female and 13 days for male. This is the first time that the life-cycle of this important malaria vector, *An. aconitus* in Central Java has been successfully established in laboratory condition.

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